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Atomic force microscopy observation and characterization of single virions and virus-like particles by nano-indentation

M Marchetti¹, GJL Wuite¹ and WH Roos^{1,2}

Structure and function of viruses are intimately related, and one of the goals in virology is to elucidate the mechanisms behind this relation. A variety of research endeavours is focused on studying these mechanisms and a relatively new technique in this field is Atomic Force Microscopy (AFM). Using AFM virions and virus-like particles can be imaged and manipulated at the single particle level. Here we review recent AFM nano-indentations studies unveiling for instance the mechanics of capsid–genome interactions, morphological changes that drive viral maturation, capsid stabilizing factors and viral uncoating. We show that in an increasing amount of literature a clear link between mechanics and infectivity is observed, which not only provides us with new fundamental insights into virology, but also provides ways to improve virus-like particles for applications in nanomedicine and nanotechnology.

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Introduction

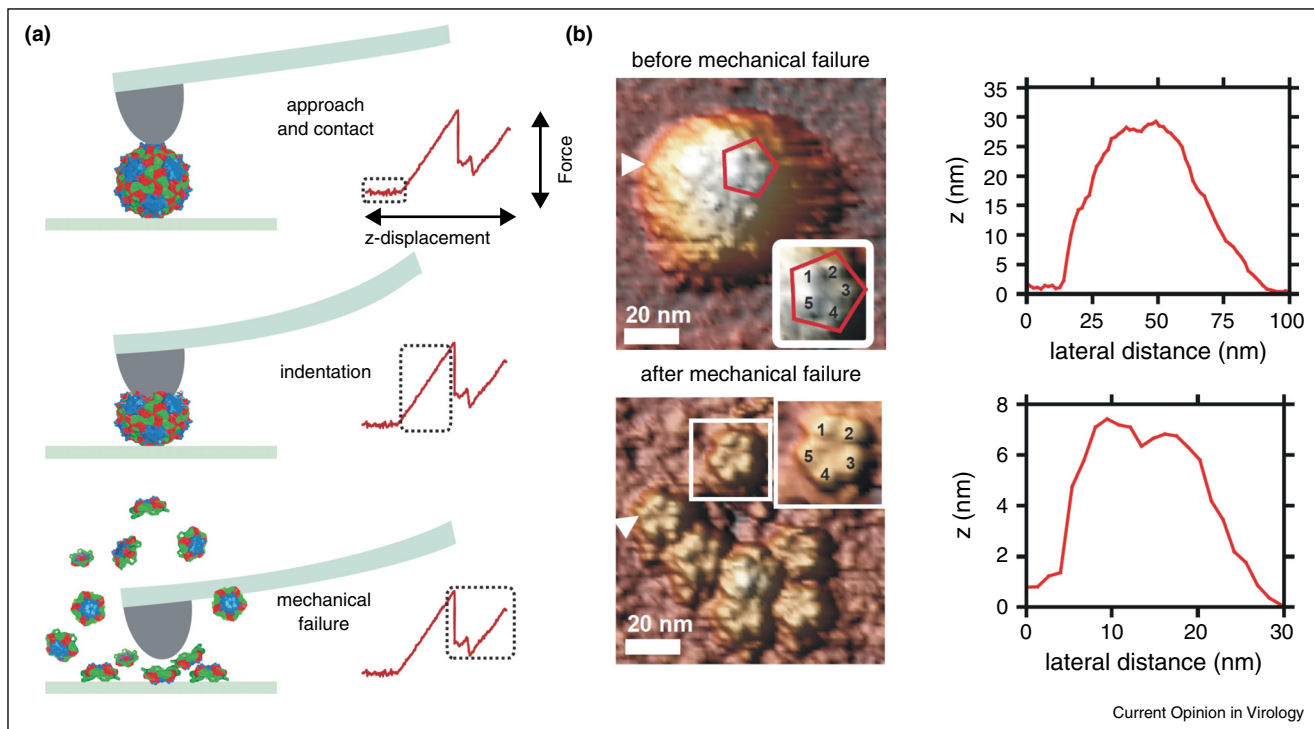
One key aim in life sciences is to explain the correlation between the molecular structure and its biological function. For example, by investigating the structural and material properties of viruses, some viral biological functions can be correlated to capsid morphology. In particular, Structural Virology provides high-resolution molecular structures [1]: it reveals capsid protein arrangements such as inter-subunit and intra-subunit organization, as well as genome–capsid interactions that are established during or

after the assembly process. Mechanical Virology, on the other hand, quantifies capsid mechanical and material properties and investigates its relation to structural changes and the virus self-assembly process [2]. These two Physical Virology branches, together with biological, biochemical and chemical approaches, create a deeper understanding of how viral proteins and capsomers are able to self-assemble in highly ordered shells, and how conformational changes inside the capsid are related to genome packaging or release [3]. These key insights not only elucidate the biology of viruses, but also allow to manipulate viruses and to create virus like-particles with numerous applications both in nanomedicine [4] and nanotechnology [5]. This review provides an overview of recent studies on the material properties of virions and virus-like particles. The main technique for these studies is Atomic Force Microscopy (AFM) imaging and manipulation.

AFM nano-indentation approach

AFM force spectroscopy allows the investigation of the nano-mechanical properties of individual molecules and particles under near-to physiological conditions [6,7]. One of the key strengths of AFM is that it allows for imaging as well as manipulation of the biological samples [8–10]. To study viral particles typically first an image is recorded by scanning the AFM cantilever over the virus covered-surface. Afterwards, the AFM cantilever is directed towards the centre of one of the viruses. Next the virus is indented and its response is analyzed as a function of the indentation (**Figure 1**). The recorded force–distance curve shows how the force changes during the indentation cycle. The initial part of the force–indentation curve is often approximately linear which permits the easy quantification of the virus spring constant (stiffness). However, in more elaborate analyses also non-linear effects can be taken into account [11]. To describe the viral material properties independent from particle geometry, size and shell thickness, the Young's modulus E is often used [8]. The Young's modulus can be approximated by ($k = \alpha(Eh^2/R)$), with the virus spring constant k , the radius R and shell thickness h . Although k is extracted from the force–indentation curve, an average value for R and h can be extracted from the virus PDB structure. The proportionality factor α has been shown to be roughly 1 and can therefore be omitted [12]. In above approximation the capsid is idealized as a homogenous material with constant radius and shell thickness. Of course this is not a realistic description of its actual structure. The heterogeneity of a viral shell can be taken

Figure 1



AFM nanoindentation. (a) Schematic of experimental approach with force-indentation curves. (b) Triatoma virus before and after failure. Both before and after failure the pentameric substructures of which the capsid is built up from can be observed. Source: Modified from Ref. [36*] with permission.

into account by more elaborate approaches using computational methods such as (coarse grained) molecular dynamics simulations, normal mode analysis, elastic network models and finite element modelling [13–17]. Another parameter to characterize material properties of a virus is the maximum force it can resist before it deforms irreversibly (often called breaking force). Repeated force indentations (or alternatively repetitive imaging at low imaging force) allows quantification of the virus resistance to mechanical fatigue and sometimes show progressive dismantling of its structure (Figure 1).

Mechanical properties of viruses

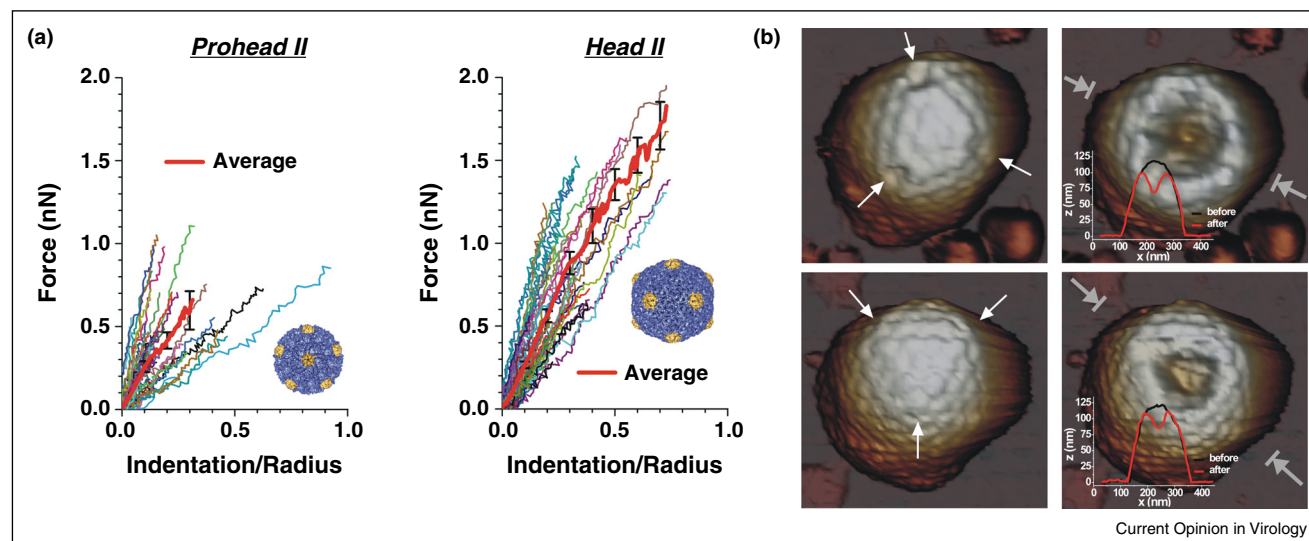
In the following we will discuss recent literature on probing the mechanical properties of viruses. For a review of older literature on AFM manipulation experiments on viruses, please see Refs. [2,3,18,19].

Mechanics of maturation

Capsid maturation is the process of concerted conformational changes of the already formed viral protein shell, resulting in the formation of a virion which is ready for infection. Bacteriophage capsid maturation often involves large structural transitions [20]. Bacteriophage HK97 is a model system for maturation studies and the different steps of the maturation pathway are well described from a

structural point of view [21,22]. Recently the mechanics of this process was also studied [17]. HK97 particles self-assemble into the immature Prohead I structure, which is a metastable particle in which the gp5 capsid protein still includes the Δ -domain. The N-terminal Δ -domain, which has a similar function to scaffolding proteins in other viruses, is subsequently cleaved off by the encapsidated protease. This leads to the formation of strong three-fold interactions between the hexamers. AFM nano-indentation has shown that this transition to Prohead-II leads to much more rigid particles. In particular the spring constant increases from 0.018 N/m to 0.12 N/m in this maturation step. Next, in the maturation process, expansion and covalent crosslinking occurs and the Head II particle is formed. It was shown by AFM that this results in a major strengthening and stabilization of the capsid (Figure 2a). It occurs concomitantly in three independent ways: increasing the capsid's Young's modulus from 0.3 GPa to 1 GPa, increasing the breaking force from 0.56 nN to 0.9 nN, as well increasing the resistance against material fatigue. This study showed for the first time that maturation of bacteriophages goes hand-in-hand with a mechanical stabilization of the particles, fitting with the requirements of the virus to be soft and easily adaptable during self-assembly (to correct for mistakes) and strong after maturation (to withstand the

Figure 2



(a) Force indentation curves until failure of bacteriophage HK97. It can be seen that the Prohead II shows both a softer response as well as a lower break force than Head II. Modified from Ref. [17] with permission. **(b)** Pentonless (top) and intact (bottom) Herpes Simplex Virus capsids. Both are shown before (left) and after (right) failure. The white arrows indicate the vertex positions, the height profiles are taken along the grey arrows. Modified from Ref. [32] with permission.

internal pressure of the packaged DNA and external hostile environments).

In another study the mechanical changes of bacteriophage λ during its maturation pathway were investigated [23]. Phage λ proteins self-assemble into a procapsid particle, which is a particle with weak non-covalent interactions between the protein subunits. To withstand the pressure (tens of atmospheres [24]) generated during genome packaging the particles need to be strengthened. During the packaging process hydrophobic binding sites for the gpD cementing proteins become exposed on the capsid surface. AFM nano-indentation experiments revealed that a major capsid reinforcement occurred upon addition of gpD. Both the stiffness increased (from 0.12 N/m for the procapsid to 0.2 N/m for the gpD-decorated capsids) as well as the breaking force (from 0.48 to 0.88 nN). Moreover fatigue experiments showed how gpD decorated phage λ are more stable to multiple external small perturbations than particles without gpD. This result shows how not only covalent cross-linking, as occurs during HK97 maturation, but also the addition of non-covalently bound cementing proteins can stabilize protein cages during maturation. Bacteriophage T7 follows again another maturation pathway where neither covalent cross-links nor additional proteins are added. Following a nano-indentation approach it was shown that despite the structural changes (expansion and thinning of the shell) upon maturation, the spring constant of the thinner and larger mature shells was not lower than that of the procapsids, but the same [25]. This already indicated that some sort of strengthening must

have occurred during maturation and this was confirmed by mechanical fatigue measurements showing that proheads are more fragile than the mature capsids.

As opposed to bacteriophages, Human Immunodeficiency virus type 1 (HIV-1) shows a major softening during its maturation transition. During maturation, the viral Gag protein is cleaved into the structural proteins: matrix, capsid and nucleocapsid. These protease-mediated morphological changes during HIV-1 maturation results in a 'stiffness switch' in the particles. In particular, it was reported that the viral envelope protein, characterized by a long cytoplasmic tail (CT) which interacts with the matrix region of Gag polyprotein subunits, is responsible for the stiffness reduction during maturation [26,27]. It turns out that the presence of the CT domain causes the relatively large stiffness of immature HIV-1. Mature particles were measured to have a stiffness of ~ 0.2 N/m, while the 14-fold stiffer immature particles have a spring constant of ~ 3 N/m. However, immature particles without the CT-domain showed a largely decreased stiffness, approaching that of mature particles. Next, the entry efficiency of the particles was analyzed in relation to the particle stiffness. The mature soft particles enter cells efficiently, whereas the immature stiff particles do not enter. However, for immature mutants lacking the CT-domain, that is, immature particles with a stiffness roughly similar to that of the mature particles, a concomitant increase in entry activity was observed. Thus HIV-1 cell entry is inversely correlated to the stiffness of the viral particles. This revealed that not the surface chemistry, but the physical stiffness of the viral particle regulates cell

entry. Herewith a direct link between viral entry efficiency and viral mechanics was shown for the retroviral HIV-1 particles.

Mechanics of uncoating

In *in vitro* experiments on viral uncoating the environmental conditions in the host at the specific stages of uncoating can be partially reconstructed to determine the influence of these conditions. In this regards, AFM nano-indentation experiments revealed how the structural uncoating of Influenza Virus could possibly occur in a two-step process [28,29]. These two steps correspond to the different pH values associated with the early and late endosome environments, as was concluded from measurements on the pH-dependent stiffness of Influenza A viruses. A first softening step would then occur in the early endosomes (pH 7.4–6.0) associated with a softening of the glycoprotein spikes layer. A second softening would then take place in the late endosomes (pH 6.0–5.5), where the M1 protein layer disassembles and dissociates from the lipid envelope, resulting in an irreversible softening. The first pH change starts the acidification of the viral lumen and initiates the structural changes of the infection process, which is completed in the late endosome with viral uncoating and genome release.

Virus–host interactions are often initiated by cell surface receptors which can strongly influence capsid mechanical properties and initiate the disassembly process. This was for instance shown by AFM nano-indentation experiments probing the effect of integrin binding on Adenovirus stability [30]. It was found that docking of integrin receptors softens the penton-bases at the vertex regions. This weakening is most likely the first step in uncoating, in which finally the penton-bases are removed completely and the genome is expelled. Next to probing the effect of integrin, also the effect of binding of the anti-microbial peptide defensin was characterized. It turned out that binding of defensin prevents capsid disassembly and genome exposure by stabilizing the virions. A different aspect of Adenovirus uncoating was studied by comparing Adeno wild-type (WT) and ts1 mutants, respectively model systems for the mature and immature Adenovirus particles. Genome uncoating was simulated by material fatigue stepwise disruption [31^{••}]. Material fatigue during low-force imaging was already reported for the Prohead-I particles of bacteriophage HK97, where it was shown how capsomers are expelled out of the capsid during imaging with an imaging force of 100 pN [17]. For Adenovirus real-time measurements of mechanical fatigue stepwise disruptions revealed the sequential dissociation of pentons. A clear difference in disruption dynamics of mature and immature particles was observed. The mature particles are more fragile and unstable than immature particles, probably due to the weaker DNA–protein interactions in the mature particles. It was previously shown that the vertices of icosahedral viral particles are under

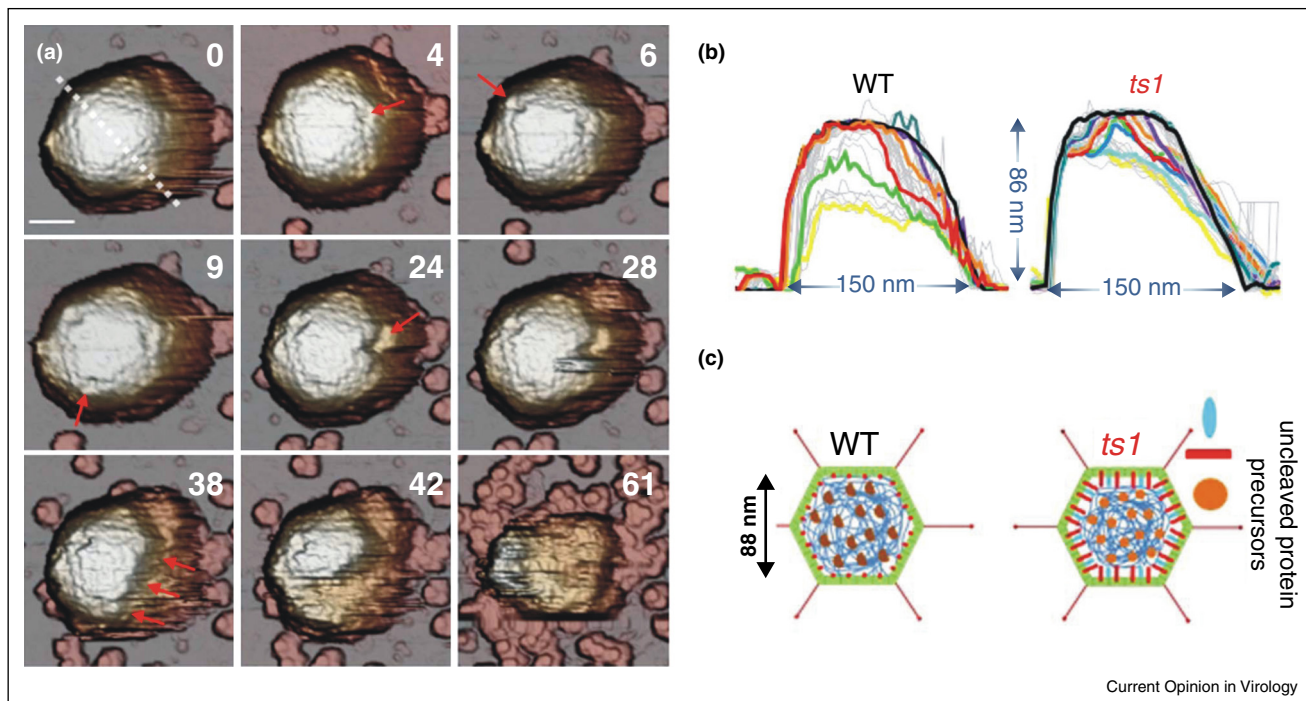
pre-stress [32] (Figure 2b), and therefore the pentons are the first capsomers to be removed when a particle is under stress. This was confirmed for the Adeno viral particles where also the pentons popped off first after the gentle mechanical perturbations (Figure 3a,b). Additionally it was reported that the state of condensation of the genomic core has a major impact on Adenovirus stability (Figure 3c). The differences in condensation states of mature and immature Adenovirus particles were corroborated by a combined AFM fluorescence study in which the expansion of the genomic core during AFM induced uncoating was monitored by fluorescence microscopy [33[•]]. Combining above experiments shows that the biology of Adeno virus infection has a strong mechanical component in which immature particles are most resilient, mature particles are already less resilient and the action of integrin binding loosens the pentons even more, in order to allow genome uncoating.

Capsid stabilizing and destabilizing interactions

Building onto the success of combining AFM nano-indentation with native mass spectrometry [34,35], the assembly, stability and uncoating of the picorna-like Triatoma virus (TrV) was investigated [36^{••}]. First, the pH dependent stability of the virion and the empty capsid was studied by mass spectrometry as well as the topology of the assembly of VP1, VP2 and VP3 units into the capsid. Next, using nano-indentation the virion was mechanically compared to empty capsids. At neutral pH the virion was found to be both stiffer ($k = 1.46$ N/m vs. $k = 0.43$ N/m) as well as more resistant to higher forces than the capsid. This observation suggested that the large amount of ssRNA genome (9 knt) is stabilizing and reinforcing the capsid at neutral pH due to non-specific interactions of the RNA with the inner capsid wall [37]. Raising the pH reverses this effect and results in a significantly weakened virion. Based on the mass spectrometry and AFM data a model was proposed in which the genome has a dual role: early in the viral life-cycle at neutral pH the genome stabilizes the virion but when the pH is increased as occurs in the hindgut of the insect host of TrV, the stabilizing interactions within the capsid are reduced which in turns supports the triggering of the release of the genomic content.

In Minute Virus of Mice (MVM) the ssDNA genome can stabilize the viral capsid [38]. Figure 4 shows the binding sites of the DNA at the inner capsid wall and the icosahedral orientation along which the strongest reinforcement is observed. Recently, also the relation between DNA-mediated stiffening and thermally induced deactivation of MVM was investigated [39]. Using a series of mutants with partially impaired DNA–capsid interactions, it was shown how virus inactivation rate constants were inversely proportional to the stiffness of the particles. The DNA mediated particles strengthening was suggested to have a biological function to increase

Figure 3



Strength of Adenovirus particles. **(a)** Array of images showing the stepwise disruption of wt particles. Numbers signify how often the particle was imaged. **(b)** Height progression of wt and *ts1* particles over time, showing the increased strength of *ts1*. **(c)** Difference in genome compaction between both particle types.

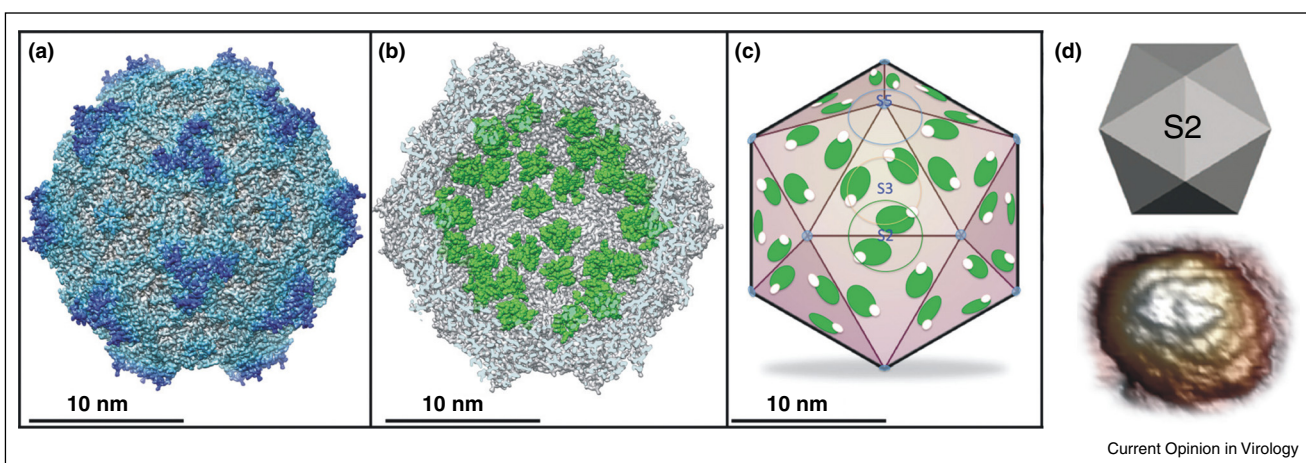
Source: Modified from Ref. [31**] with permission.

survival rate of MVM particles under thermal stress in hostile environments.

Another study that explored genome–capsid interactions was conducted on Brome Mosaic virus (BMV) [40]. The

BMV genome is divided over three subsets of virions which were previously thought to be indistinguishable. Notably, it has now been reported that they do behave mechanically different. This difference relates to the sensitivity to pH-dependent swelling transitions, virion

Figure 4



Genome–capsid interactions in Minute Virus of mice. **(a)** Reconstruction of capsid. **(b)** Inside view showing specific genome (green) interactions with the capsid interior. **(c)** Schematic showing the prevalence of interactions at the 2-fold symmetry site (S2). **(d)** Schematic and AFM image of capsid absorbed on S2.

Source: Modified from Ref. [39] with permission.

stiffness and disassembly behaviour. It was suggested that these differences affect the expression of the RNAs and the timing of the infection process. A related virus, Cowpea Chlorotic Mottle Virus (CCMV), has been a model system for many viral studies [41–43]. In a recent study, the pH dependent swelling behaviour of CCMV was scrutinized showing that the increase in diameter lead to the softening of the particles at increasing pH (from 0.195 N/m at pH 4.8 to 0.046 N/m at pH 7.5) [44], revealing yet another capsid (de)stabilizing mechanism.

Herpes Simplex Virus type 1 (HSV-1) encapsidates its dsDNA genome inside a $T = 16$ icosahedral capsid of 125 nm in diameter. The mechanical properties of empty capsids as well as ones with the scaffold protein or DNA have been investigated. Moreover, the destabilizing action of penton removal was determined [32,45,46]. Following a suggestion that minor capsid proteins could have a major effect on capsid mechanical stability, deletion mutants lacking the minor capsid protein UL25 were probed by AFM [47]. Indeed these mutants turned out to be weaker than the wild type capsids. It is expected that deletion of the minor capsid protein UL17, that together with UL25 forms the capsid vertex specific component (CVSC) [48], will have the same destabilizing effect.

Finally, the VP3 protein of Avian Infectious Bursal Disease Virus (IBDV) acts as scaffolding protein during assembly and as dsRNA-binding protein in the genome-packaging process. Its role as multifunctional protein in capsid stabilization was recently verified by AFM mechanical fatigue experiments which were suggested to mimic the virus disassembly pathway leading to infection [49]. It was reported that VP3 proteins can reinforce the viral capsid, but only when it is bound to the dsRNA genome of IBDV. In absence of the viral genome, no significant stabilization was observed. This strengthening, which originates from nucleic acid-protein interactions, shows again how proteins can have a stabilizing effect on virions and thereby possibly influencing the viral life-cycle.

Conclusions

We have shown how using AFM nano-indentation we are not only yielding insights into the construction principles nature is using for self-assembly of viral particles, we also start to obtain a picture of the importance of mechanics in the viral life cycle. Besides the experimental nano-indentation methods as reviewed here, there are also many recent viral AFM imaging studies [50,51] as well as modelling and simulations of viral mechanics [16,52–55] published. All these new insights regarding virus structure and mechanics provide deep fundamental virology knowledge. But it also facilitates the applications of viral cages as carriers in drug delivery [56], as vaccines [57], and in nanotechnology [58–60], causing a small shift

from viral particles as harmful agents towards particles that can be turned for good use.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Rossmann MG: **Structure of viruses: a short history.** *Quart Rev Biophys* 2013, **46**:133-180.
2. Roos WH, Bruinsma R, Wuite GJL: **Physical virology.** *Nat Phys* 2010, **6**:733-743.
3. Mateu MG: *Structure and Physics of Viruses*. Springer; 2013.
4. Wen AM et al.: **Design rules for nanomedical engineering: from physical virology to the applications of virus-based materials in medicine.** *J Biol Phys* 2013, **39**:301-325.
5. Singh P, Gonzalez MJ, Manchester M: **Viruses and their uses in nanotechnology.** *Drug Dev Res* 2006, **67**:23-41.
6. Kasas S, Longo G, Dietler G: **Mechanical properties of biological specimens explored by atomic force microscopy.** *J Phys D-Appl Phys* 2013, **46**.
7. Kurland NE, Drira Z, Yadavalli VK: **Measurement of nanomechanical properties of biomolecules using atomic force microscopy.** *Micron* 2012, **43**:116-128.
8. Roos WH: **How to perform a nanoindentation experiment on a virus.** *Methods Mol Biol (Clifton, N.J.)* 2011, **783**:251-264.
9. van Rosmalen MGM, Roos WH, Wuite GJL: **Material properties of viral nanocages explored by atomic force microscopy.** *Methods Mol Biol (Clifton, N.J.)* 2015, **1252**:115-137.
10. de Pablo PJ: **Atomic force microscopy of viruses.** *Sub-cellular Biochem* 2013, **68**:247-271.
11. Gibbons MM, Klug WS: **Influence of nonuniform geometry on nanoindentation of viral capsids.** *Biophys J* 2008, **95**:3640-3649.
12. Michel JP et al.: **Nanoindentation studies of full and empty viral capsids and the effects of capsid protein mutations on elasticity and strength.** *Proc Natl Acad Sci U S A* 2006, **103**:6184-6189.
13. Zink M, Grubmüller H: **Primary changes of the mechanical properties of southern bean mosaic virus upon calcium removal.** *Biophys J* 2010, **98**:687-695.
14. Arkhipov A et al.: **Elucidating the mechanism behind irreversible deformation of viral capsids.** *Biophys J* 2009, **97**:2061-2069.
15. Kononova O et al.: **Fluctuating nonlinear spring model of mechanical deformation of biological particles.** *PLoS Comput Biol* 2016, **12**.
16. Cieplak M, Robbins MO: **Nanoindentation of 35 virus capsids in a molecular model: relating mechanical properties to structure.** *PLoS One* 2013, **8**.
17. Roos WH et al.: **Mechanics of bacteriophage maturation.** *Proc Natl Acad Sci U S A* 2012, **109**:2342-2347.
18. Baclayon M, Wuite GJL, Roos WH: **Imaging and manipulation of single viruses by atomic force microscopy.** *Soft Matter* 2010, **6**:5273-5285.
19. Mateu MG: **Mechanical properties of viruses analyzed by atomic force microscopy: a virological perspective.** *Virus Res* 2012, **168**:1-22.

20. Veesler D, Johnson JE: **Virus maturation.** *Ann Rev Biophys* 2012, **41**:473-496.
21. Cardone G *et al.*: **Metastable intermediates as stepping stones on the maturation pathways of viral capsids.** *Mbio* 2014, **5**.
22. Hendrix RW, Johnson JE: **Bacteriophage HK97 capsid assembly and maturation.** *Viral Mol Mach* 2012, **726**:351-363.
23. Hernando-Perez M *et al.*: **Cementing proteins provide extra mechanical stabilization to viral cages.** *Nat Commun* 2014, **5**.
24. Fuller DN *et al.*: **Measurements of single DNA molecule packaging dynamics in bacteriophage lambda reveal high forces, high motor processivity, and capsid transformations.** *J Mol Biol* 2007, **373**:1113-1122.
25. Hernando-Perez M *et al.*: **The interplay between mechanics and stability of viral cages.** *Nanoscale* 2014, **6**:2702-2709.
26. Kol N *et al.*: **A stiffness switch in human immunodeficiency virus.** *Biophys J* 2007, **92**:1777-1783.
27. Pang H-B *et al.*: **Virion stiffness regulates immature HIV-1 entry.** *Retrovirology* 2013, **10**.
 • Study on HIV showing that stiffness switch is trigger for entry, revealing the direct link between mechanics and infectivity.
28. Greber UF: **How cells tune viral mechanics-insights from biophysical measurements of influenza virus.** *Biophys J* 2014, **106**:2317-2321.
29. Li S *et al.*: **pH-controlled two-step uncoating of influenza virus.** *Biophys J* 2014, **106**:1447-1456.
30. Snijder J *et al.*: **Integrin and defensin modulate the mechanical properties of adenovirus.** *J Virol* 2013, **87**:2756-2766.
31. Ortega-Esteban A *et al.*: **Monitoring dynamics of human adenovirus disassembly induced by mechanical fatigue.** *Scient Rep* 2013, **3**.
 • Seminal study showing how by repeated imaging at low force pentons are released first and visualizing how gradually the particle disintegrates. Thereby it introduces a new approach to characterize viral strength.
32. Klug WS, Roos WH, Wuite GJL: **Unlocking internal prestress from protein nanoshells.** *Phys Rev Lett* 2012, **109**.
33. Ortega-Esteban A *et al.*: **Fluorescence tracking of genome release during mechanical unpacking of single viruses.** *ACS Nano* 2015, **9**:10571-10579.
 • Clear step forward in technology by a combined AFM and fluorescence imaging approach of virion disintegration and genome release in Adenovirus.
34. Uetrecht C *et al.*: **High-resolution mass spectrometry of viral assemblies: molecular composition and stability of dimorphic hepatitis B virus capsids.** *Proc Natl Acad Sci U S A* 2008, **105**:9216-9220.
35. Baclayon M *et al.*: **Prestress strengthens the shell of norwalk virus nanoparticles.** *Nano Lett* 2011, **11**:4865-4869.
36. Snijder J *et al.*: **Probing the biophysical interplay between a viral genome and its capsid.** *Nat Chem* 2013, **5**:502-509.
 • Study exemplifying the strength of a combined AFM and mass spectrometry approach for virus characterization. Here this approach reveals capsid protein topology and a dual stabilizing/destabilizing role of the genome in Triatoma Virus.
37. Agirre J *et al.*: **Cryo-electron microscopy reconstructions of triatoma virus particles: a clue to unravel genome delivery and capsid disassembly.** *J Gen Virol* 2013, **94**:1058-1068.
38. Carrasco C *et al.*: **DNA-mediated anisotropic mechanical reinforcement of a virus.** *Proc Natl Acad Sci U S A* 2006, **103**:13706-13711.
39. Castellanos M, Carrillo PJP, Mateu MG: **Quantitatively probing propensity for structural transitions in engineered virus nanoparticles by single-molecule mechanical analysis.** *Nanoscale* 2015, **7**:5654-5664.
40. Vaughan R *et al.*: **The tripartite virions of the brome mosaic virus have distinct physical properties that affect the timing of the infection process.** *J Virol* 2014, **88**:6483-6491.
41. Klug WS *et al.*: **Failure of viral shells.** *Phys Rev Lett* 2006, **97**.
42. Snijder J *et al.*: **Probing the impact of loading rate on the mechanical properties of viral nanoparticles.** *Micron* 2012, **43**:1343-1350.
43. Luque D *et al.*: **Self-assembly and characterization of small and monodisperse dye nanospheres in a protein cage.** *Chem Sci* 2014, **5**:575-581.
44. Wilts BD, Schaap IAT, Schmidt CF: **Swelling and softening of the cowpea chlorotic mottle virus in response to pH shifts.** *Biophys J* 2015, **108**:2541-2549.
45. Liashkovich I *et al.*: **Exceptional mechanical and structural stability of HSV-1 unveiled with fluid atomic force microscopy.** *J Cell Sci* 2008, **121**:2287-2292.
46. Roos WH *et al.*: **Scaffold expulsion and genome packaging trigger stabilization of herpes simplex virus capsids.** *Proc Natl Acad Sci U S A* 2009, **106**:9673-9678.
47. Sae-Ueng U *et al.*: **Major capsid reinforcement by a minor protein in herpesviruses and phage.** *Nucleic Acids Res* 2014, **42**:9096-9107.
48. Toropova K *et al.*: **The Herpes Simplex Virus 1 UL17 protein is the second constituent of the capsid vertex-specific component required for DNA packaging and retention.** *J Virol* 2011, **85**:7513-7522.
49. Mertens J *et al.*: **A protein with simultaneous capsid scaffolding and dsRNA-binding activities enhances the birnavirus capsid mechanical stability.** *Scient Rep* 2015, **5** 13486-13486.
50. Cartagena A *et al.*: **Mapping in vitro local material properties of intact and disrupted virions at high resolution using multi-harmonic atomic force microscopy.** *Nanoscale* 2013, **5**:4729-4736.
51. Chen S-WW *et al.*: **Nanoscale structural features determined by AFM for single virus particles.** *Nanoscale* 2013, **5**:10877-10886.
52. Roos WH *et al.*: **Squeezing protein shells: how continuum elastic models, molecular dynamics simulations, and experiments coalesce at the nanoscale.** *Biophys J* 2010, **99**:1175-1181.
53. Kononova O *et al.*: **Structural transitions and energy landscape for cowpea chlorotic mottle virus capsid mechanics from nanomanipulation in vitro and in silico.** *Biophys J* 2013, **105**:1893-1903.
54. Boyd KJ *et al.*: **Stability of norwalk virus capsid protein interfaces evaluated by in silico nanoindentation.** *Front Bioeng Biotechnol* 2015, **3** 103-103.
55. Polles G *et al.*: **Mechanical and assembly units of viral capsids identified via quasi-rigid domain decomposition.** *PLoS Comput Biol* 2013, **9**.
56. Molino NM, Wang S-W: **Caged protein nanoparticles for drug delivery.** *Curr Opin Biotechnol* 2014, **28**:75-82.
57. Gregory AE, Titball R, Williamson D: **Vaccine delivery using nanoparticles.** *Front Cell Infect Microbiol* 2013, **3**:p13.
58. Aniagyei SE *et al.*: **Self-assembly approaches to nanomaterial encapsulation in viral protein cages.** *J Mater Chem* 2008, **18**:3763-3774.
59. de la Escosura A, Nolte RJM, Cornelissen JJLM: **Viruses and protein cages as nanocontainers and nanoreactors.** *J Mater Chem* 2009, **19**:2274-2278.
60. Uchida M *et al.*: **Use of protein cages as a template for confined synthesis of inorganic and organic nanoparticles.** In *Protein Cages*. Edited by Orner BP. Springer; 2015:17-25.